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The basic helix-loop-helix transcription factors, E12 and E47 (products of the E2A gene), have been implicated to have a role as tumor suppressors. Conversely, the inhibitors of E12 and E47, the Id proteins, have been shown to stimulate cell growth, and cause T cell lymphoma when overexpressed in the T cell lineage. This project is to test the hypothesis that Id-1 overexpression in mammary glands may contribute to the development of breast cancer. In this first year of the funding period, we have established transgenic mouse lines, pYY-Id1-30 and pYY-Id1-59, in which Id1 is expressed under the control of MMTV LTR. The biological effects of Id1 overexpression on normal mammary gland development and tumorigenesis are being investigated. We have also found that Id1 is overexpressed in two of breast cancer cell lines, T47D and MDA-MB-231. Consequently, the transcriptional activity of E47 (or E12) is extremely low even though the proteins are readily detectable. We are currently testing if restoration of the transcription activity of E47 could lead to growth arrest of the breast cancer cells. These studies may establish a tumor suppressing function of E2A proteins in mammary glands, and shed light on the molecular mechanisms concerning tumorigenesis in breast tissues.

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FOREWORD

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(5) INTRODUCTION

The E2A gene is ubiquitously expressed and encodes two alternatively spliced products, E12 and E47, which are potent transcription factors containing the basic helix-loop-helix (bHLH) domain for DNA binding and dimerization (Murre et al., 1989). E12 and E47 form homodimers or heterodimers with other tissue-specifically expressed bHLH transcription factors to bind to a consensus sequence called E box. These bHLH proteins have been shown to play crucial roles in the differentiation of a variety of cell types such as the lymphocytes, muscle cells, pancreatic cells, and neuronal cells (Zhuang et al., 1994; Bain et al., 1994; Weitraub. et al., 1991; Naya et al., 1995; Lee et al., 1995). In addition to dimerizing with the bHLH proteins and binding to DNA, E12 and E47 can also form heterodimers with the Id proteins (Id1-4), which has the HLH domain for dimerization but not the basic region for DNA binding. Therefore, E12 and E47 can be sequestered into inactive complexes and their function as transcription factors can be inhibited. Indeed, it has been shown that overexpression of Id proteins blocks the differentiation of many cell lineages including the B and T lymphoid, muscle, neuronal, adipose and mammary epithelial cells (Sun, 1994; Kim et al., 1999; Jen et al., 1992; Moldes et al., 1997; Desprez et al., 1995).

Apart from the differentiation function of E2A gene products, they have also been implicated to have a role as tumor suppressors. In NIH3T3 fibroblasts, overexpression of E47 or incubation with Id antisense oligonucleotides arrests cell cycle at the G1 to S phase transition (Peverali, et al., 1994; Hara et al., 1994; Hara et al., 1994). We have shown that E2A can activate transcription of the gene encoding cyclin dependent kinase inhibitor, $p21^{CIP}$, through the E box sequences located in the promoter region of the p21 gene (Prabhu et al., 1997). In mice, disruption of the E2A gene (Bain et al., 1996; Yan et al., 1997) or overexpression of the Id-1 inhibitor in the T cell lineage (Kim et al., 1999) results in the development of T cell lymphoma at very high frequencies, thus suggesting a tumor suppressor function for the E2A proteins. Evidence implicating the tumor suppressor function of E2A have also come the studies of the TAL family of oncogenes (reviewed by Baer, 1993). The TAL proteins, including Tal1, Tal2 and Lyl1, are all bHLH proteins that can dimerize with E2A proteins and bind to E boxes. Aberrant expression of the Tal1 gene, for example, has been found in 70% of human acute lymphoblastic leukemia (T-ALL) samples. Although Tal1 can form heterodimers with E47 to bind to DNA, the heterodimers potentiate the transcription of their target genes very poorly as compared to E47 homodimers. We have shown that the poor transactivation by E47/Tal1 heterodimers is due to the incompatibility of the activation domains present in E47 and Tal1 (Park and Sun, 1998). We have then constructed a chimeric protein, E-T/2, which contains the N-terminus of E47 with the two known activation domains and the C-terminus of Tal1 including the bHLH domain for DNA binding and dimerization. The E-T/2 chimeric protein can activate transcription as heterodimers with E47 even more potently than E47 homodimers. To test the hypothesis that the normal function of E47 is inhibited in T-ALL cells as a result of aberrant Tal1 expression, we have introduced the E-T/2 construct into the Jurkat T-ALL cell line, in which the majority of E47 proteins are bound to Tal1 (Park et al., 1999). By competing with Tal1 to bind to endogenous E47, E-T/2 was able to restore the transcription activity of E47. Because Tal1 does not form homodimers, E-T/2 would not form homodimers to activate transcription. The activity of E-T/2 is therefore limited to a near physiological level by the availability of endogenous E2A proteins. As a result of the restoration of E47 activity in Jurkat cells, the growth of Jurkat cells was dramatically inhibited, and apoptosis also occurred. These result would suggest that the mechanism by which Tal1 causes T cell leukemia might involves the inhibition of the tumor suppressing function of E2A proteins like E47. The principles and strategies of these experiments have been employed in our studies concerning breast cancer.

Regarding mammary epithelial cells, overexpression of Id-1 has been found to block the induced differentiation of a mouse mammary epithelial cell line, SCp2, that has been exposed to basement membrane and lactogenic hormones (Desprez et al., 1995). Moreover, these Id1 expressing SCp2 cells showed invasion of the basement membrane and resumption of cell proliferation. Recently, the same researchers have shown that Id-1 overexpression stimulates the expression of two novel polypeptides with gelatinase activities. It was therefore proposed that Id-1 expression may be related to the metastasis of breast cancer (Desprez et al., 1998). We have obtained preliminary evidence that Id-1 and Id-2 appear to be overexpressed in a fraction of breast cancer samples by using RT-PCR analyses. Based on these data, we have hypothesized that overexpression of Id proteins may lead to the inhibition of E2A function, which may then interfere with the normal process of mammary cell differentiation and tumor suppression. To test this hypothesis, we had designed experiments to be carried out in Year 1, which examine the effect of Id-1 overexpression in transgenic mice and the effect of the restoration of E2A function in breast cancer cell lines found to express high levels of Id-1. The progress in these experiments are outlined below.

(6) BODY

1. Examination of MMTV-Id-1 transgenic mice

To test the effect of Id proteins on tumorigenesis and mammary gland development, we have generated transgenic mice carrying the Id-1 gene under the control of the mouse mammary tumor virus (MMTV) long terminal repeat (LTR). The Id1 cDNA was cloned into a modified MMTV transgenic vector obtained from Dr. B. Weinstein (Columbia University), which has been shown to direct high levels of expression in mammary tissues and low levels of expression in salivary gland and some male reproductive tissues (Yao and Weinstein, personal communication). We initially obtained six independent transgenic lines, of which two lines did not give any progenies, one line showed no expression, and three lines exhibited some levels of expression of the transgene upon preliminary analysis. Figure 1 shows a more comprehensive analysis of transgene expression in one of the lines, pYY-59. By six weeks of age, expression of the transgene is clearly detectable but is markedly increased by 11 weeks of age. In contrast to the transgene, endogenous Id-1 gene is expressed at a very low level at all ages.

Our effort to further analyze the biological effects of Id-1 overexpression has been significantly hindered by a major disaster of mouse hepatitis virus infection in the animal facility. After several months of delay, the infection has finally been under control, and all our transgenic mice have recently been rederived back to the pathogen-free facility. In the process, one of the MMTV-Id-1 transgenic line was lost. We therefore have two transgenic lines, pYY-Id1-30 and pYY-Id1-59, available for future studies. We are currently breeding the mice to obtain sufficient numbers of animals. A more detailed analysis of transgene expression in the pYY-30 line will be carried out once the mice become available. Expression levels at different ages will be compared between the pYY-30 and pYY-59 lines.

Despite the problems in the animal facility, we were able to examine mammary gland development in a few virgin pYY-59 mice at different ages. The mammary glands were dissected, well-spread out on glass slides, fixed in Carnoy's fixative and treated with Carmine Alum stain. Branching of the glands were examined by microscopy. An example of such analysis is shown in Figure 2. This preliminary analysis showed no significant difference in the number of branches between the wild type and transgenic liter mates. Further analysis is necessary to compare mammary gland development during pregnancy and lactation.

So far, we have not detected tumor formation in the limited number of transgenic mice we have maintained. While we are breeding a larger number of transgenic mice to monitor tumor development, we are also crossing the transgenic lines with the MMTV-Her-2/neu transgenic mice purchased from the Jackson Laboratory to determine the effect of Id-1 expression on neu2 induced tumorigenesis.

2. Examination of Id-1 and E2A gene expression in breast cancer cell lines.

To test the hypothesis that Id-1 overexpression may abolish the growth inhibitory effect of E2A and eventually leads to tumorigenesis. We have examined Id-1 and E2A gene expression in two breast cancer cell lines, T47D and MDA-MB-231, which were recently purchased from ATCC. As shown in Figure 3, Id-1 and E2A mRNA and protein levels were measured using RT-PCR and western blot assays. T47D and MDA-MB-231 cells were cultured in the presence (odd numbered lanes) and absence (even numbered lanes) of fetal calf serum for two days. Since T47D is an estrogen-dependent cell line, serum starvation of the cells was carried out in a Phenol-red-free medium. Both cell lines expressed Id-1 at a high level but serum starvation resulted in a 7-fold and 2-fold reduction in T47D and MDA-MB-231 cells. This effect of serum starvation is unlikely due to the synchronization of cells in a particular phase of the cell cycle because no difference in the percentage of cells in each phase of the cell cycle in the presence and absence of the serum was found based on the propidium iodine staining patterns. Surprisingly, despite the changes in Id-1 mRNA levels, the protein level did not alter significantly. This is in contrast to the situation in other cell types, e.g., in BaF3 pro-B cells Id-1 mRNA and protein levels both decrease dramatically within 30 minutes after IL-3 deprivation. Whether there exist any mechanisms to stabilize Id-1 remains to be investigated.

On the other hand, the levels of E47 mRNA were not affected by serum starvation but the protein levels were significantly increased in both T47D and MDA-MB-231 cells. Since serum starvation leads to growth inhibition of the cells, it is reasonable to find an increase in the level of a growth inhibitor, E47, in these cells. However, the fact that serum starvation resulted in increases in Id-1 and E47 proteins levels as compared to their mRNA levels raises a possibility that a common mechanism involving protein stabilization or reduction in protein turnover may take place upon serum starvation. Currently, we are investigating the interaction of Id-1 and E47 in these breast cancer cell lines, the DNA binding activity of E47 and its subcellular localization of the E47 and Id-1 proteins in culture conditions with or without fetal calf serum.

3. E-box-mediated transcription activity in breast cancer cell lines.

As shown in Figure 3, both E47 and Id-1 are expressed in T47D and MDA-MB-231 cells. This raises a question whether E47 is transcriptionally active, or its activity is completely inhibited by the Id-1 protein or by other unidentified mechanisms. We next tested the transcription activity by using a luciferase reporter gene controlled by 5 copies of the E box sequences recognized by E47. The results are shown in Figure 4. Transfection of the reporter alone into the two breast cancer cell lines generated very low luciferase activities, and these low activities could not be inhibited by co-transfection with a Id-1 expressing plasmid. Therefore, we conclude that in T47D and MDA-MB-231 cells, E-box-mediated transcription activity is not detectable. In contrast, when an E47 expression plasmid was co-transfected with the reporter into T47D cells, a 400-fold increase in luciferase activity was observed. More importantly, when an E-T/2 expressing plasmid was co-transfected with the reporter into T47D, a 10-fold increase of the report activity was also found. As mentioned in the introduction, E-T/2 contains the bHLH domain of Tal1, which does not form homodimers. Consequently, E-T/2 by itself would not be able to bind to DNA and activate transcription. Therefore, the transcription activity observed after E-T/2 co-transfection is likely resulted from E-T/2 heterodimers with endogenous E47. Perhaps, E-

T/2 could compete with endogenous Id-1 to dimerize with E47 and restore the transcription activity of E47. This level of activity (10-fold activation) is probably close to a physiological level because it is limited by the availability of endogenous E47. If exogenous E47 is provided along with E-T/2, the trans-activation could reach 1800-fold.

In MDA-MB-231 cells, however, co-transfection of E47 with the reporter resulted in a much smaller (150-fold) increase in luciferase activity as compared to that in T47D cells. This is not due to a lower efficiency of co-transfection because MDA-MB-231 cells can be transfected at a higher efficiency than T47D cell as judged by measuring green fluorescent protein positive cells after transfection of the GFP expressing plasmid (data not shown). Interestingly, expression of the E-T/2 protein did not lead to any activation of the reporter gene expression. This is not because the E-T/2 is incapable of activating transcription in the MDA-MB-231 cells because co-transfection of E47 and E-T/2 led to a 80-fold activation. We interpret the data to mean that in these cells, an additional mechanism might exists, which could, for example, disable the trans-activating function of endogenous E47 and thus prevent gene activation as heterodimers with E-T/2. This mechanism could also partially inactivate exogenous E47 and E-T/2, and diminish the transactivation potentially of E47 homodimers and E47/E-T/2 heterodimers.

An alternative interpretation of the results obtained in MDA-MB-231 cells could be that these cells are particular sensitive to E47 activities, and they die and detach from the plates before luciferase activities can be measured. This issue will be addressed in our ongoing experiments which test the effect of E47 and E-T/2 expression on the growth properties of the T47D and MDA-MB-231 cells. After attempts with several expression systems, we are using the tetracycline inducible expression retroviral vectors recently developed in Helen Blau's laboratory. The retroviral constructs have been prepared and cell lines are in the process of being generated.

4. Detection of Id-1 overexpression in human breast cancer samples.

As a project planned to be carried out in Year 2, we proposed to examine human breast cancer samples for Id-1 overexpression by using the in situ RNA hybridization technique. The major challenge in this experiment is that in situ hybridization has to be carried out using archived and formalin fixed tissue imbedded in paraffin. We initially used dioxigenin-labeled antisense RNA probes for hybridization followed by chromogenic detection with anti-dioxigenin antibodies directly conjugated with alkaline phosphotase, or in combination with secondary antibodies conjugated with alkaline phosphotase. After many attempts, we found that the non-specific background as judged by the signals generated by using the sense probes on these archived and formalin fixed tissues is too high for the results to be reliable. We thus switched to ^{35}S-labeled RNA probes and the background was significantly reduced. We have used sense and antisense β-actin probes as positive controls. We also tested a limited number of tissues for Id-1 expression by using sense and antisense Id-1 probes, and found a fraction of the samples overexpressing Id-1. The samples are currently being exposed to emulsion for microscopic examination. Shown in Figure 5 is a preliminary exposure to PhosphoImager, simply to demonstrate that we have worked out the technique and are in a position to analyze a large number of tissues.

(7) KEY RESEARCH ACCOMPLISHMENTS

- Id-1 transgenic mice generated, and transgene expression detected.
- Id-1 and E47 proteins both found to be expressed in T47D and MDA-MB-231 breast cancer cell lines.
- Endogenous E47 activity not detected in either of the cell lines, suggesting E47 is inhibited.
- Differences in E47 inhibition found between T47D cells and MDA-MB-231 cells as suggested by the ability of the E-T/2 protein to restore E47 activity.
- In situ RNA hybridization technique worked out for using archived and formalin-fixed tissues.

(8) REPORTABLE OUTCOMES

- manuscripts, abstracts presentations: None.
- patents and license applied for and/or issued: None.
- degrees obtained that are supported by this award: None.
- development of cell lines, tissue or serum repositories:
  Id-1 transgenic mouse lines, pYY-Id1-30 and pYY-Id1-59.
- informatics such as databases and animal models, etc.
- funding applied for based on work supported by this award: None
- employment or research opportunities applied for and/or received on experiences/training supported by this award: None.

(9) CONCLUSIONS

Studies in Year 1 of the proposal have laid the ground to test our original hypothesis, i.e., inhibition of E2A (such as E47) function by Id overexpression may contribute to breast cancer development.

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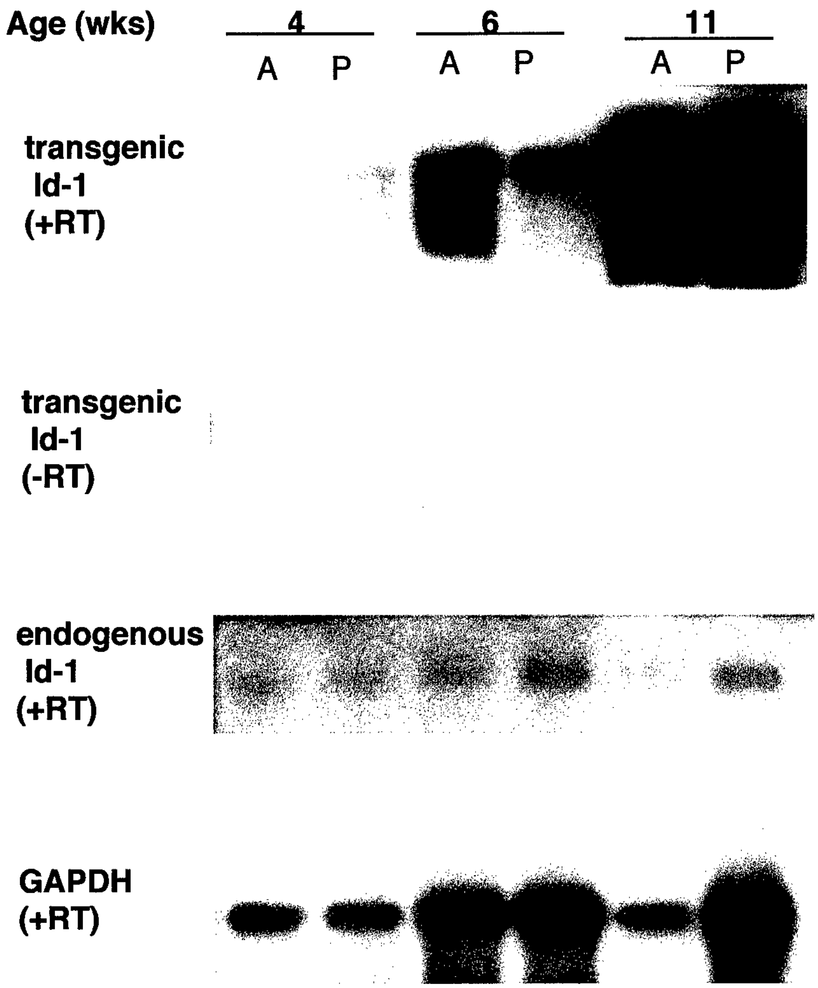


Figure 1. Expression of the Id-1 transgene in the pYY-59 transgenic mice.

Mice of the indicated ages were sacrifaced. Anterior (A) and posterior (P) mammary glands were dissected out and cut into thin slices using . RNAs were isloated by using Trizol reagent (Gibco, BRL), and treated with RNAse-free DNAse prior to reverse-transcriptase reactions. PCR reactions were carried out by using primers specific for each of the indicated transcript and RT reaction mixes incubated with (+RT) or without (-RT) reverse transcriptase as templates. PCR products were southern blotted and hybridized with probes specific for the indicated transcripts.

Wild type **pYY-Id1-59**

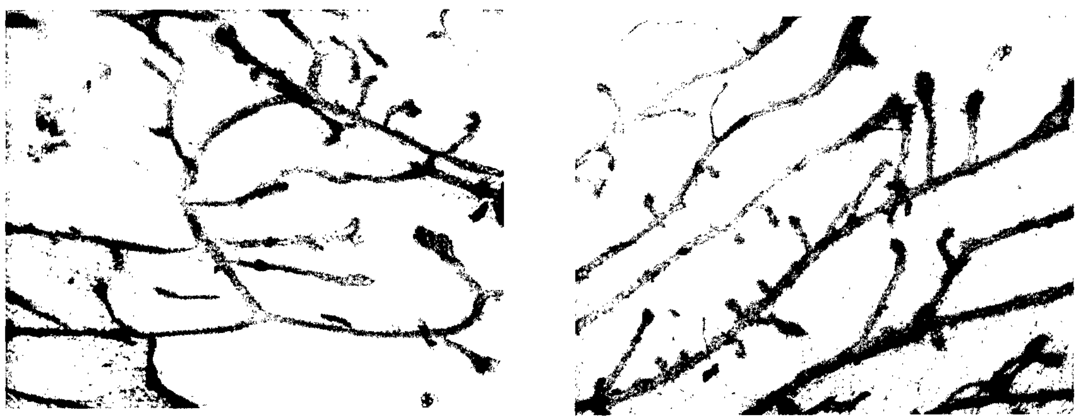


Figure 2. Branching analysis of mammary glands in the pYY-Id1-59 transgenic mice.

Six weeks old virgin wild type and transgenic liter mates were used. The staining procedure is described in detail on the web site: http://mammary.nih.gov/index.html.

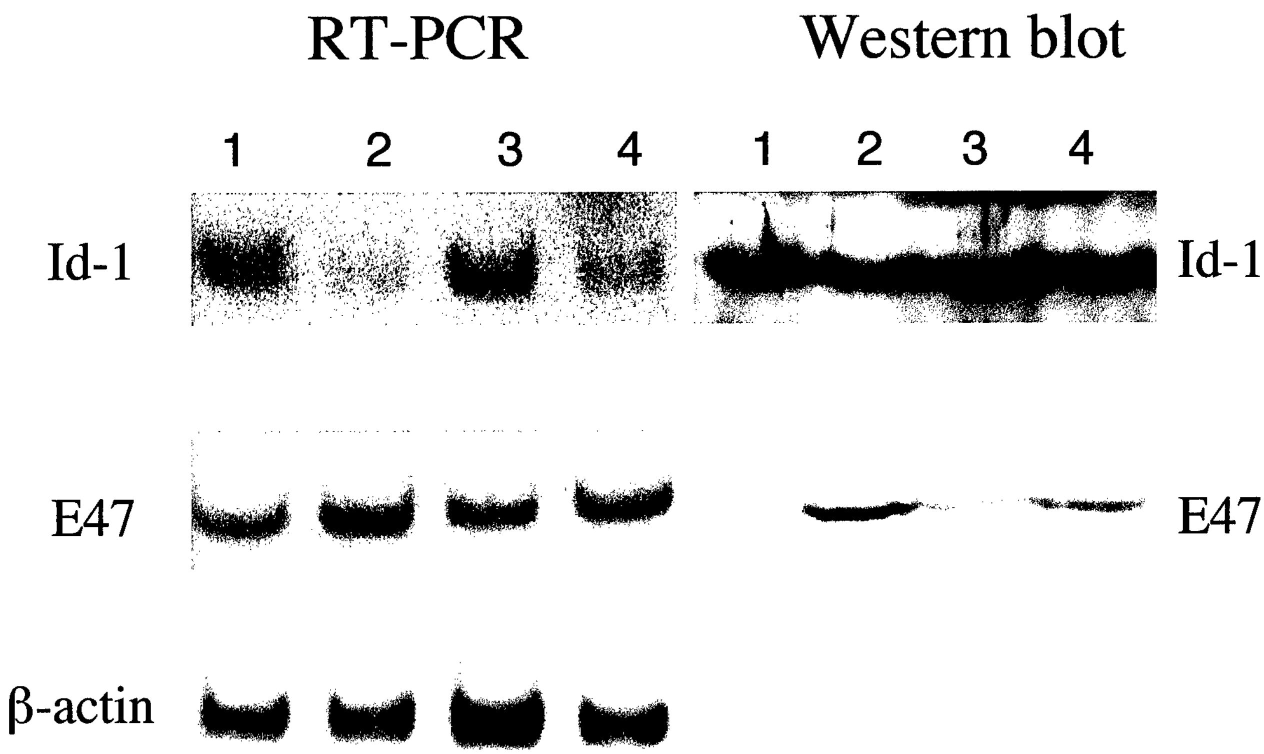
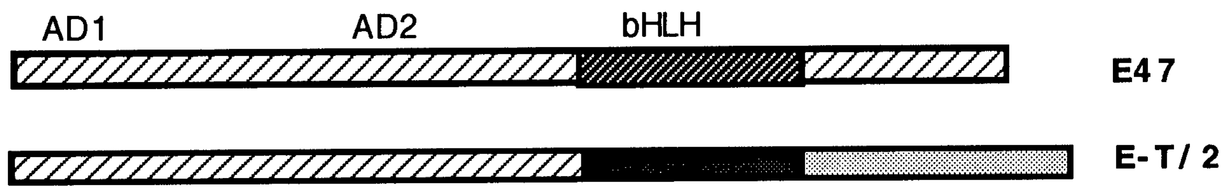


Figure 3. Id1 and E47 expression in breast cancer cell lines.

RNAs were isolated from T47D (lanes 1 and 2) and MDA-MB-231 (lanes 3 and 4) cells cultured in the prsence (lanes 1 and 3) or absence (lanes 2 and 4) of fetal calf serum. cDNAs were prepared by using MMTV-reverse transcriptase and random primers. PCRs were carried out with primers specific to the indicated transcripts for 27 cycles in the presence of ^{32}P-dATP. The PCR products were analysed on non denaturing polyacrylamide gels. The gels were then dried and exposed to PhosphoImager screens. The signals were quantified using a PhosphoImager. Signals for β-actin were used for normalization.

For Western blots, total cell lysates were prepared and protein concentrations were determined using the BCA reagents. Equal amount of protein was loaded in each lane. Polyclonal antibodies against Id-1 and E47 were purchased from Santa Cruz Biotech. Antibody binding was detected using ECL reagents.



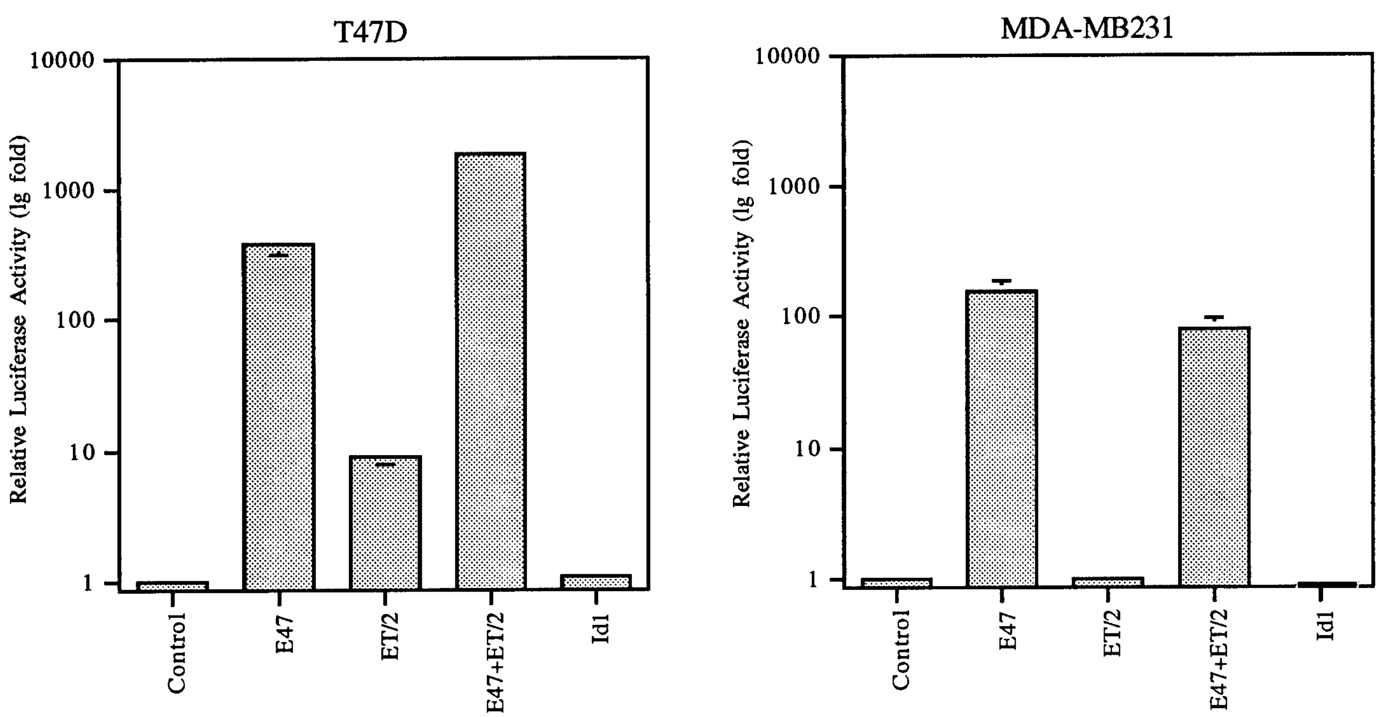


Figure 4. Transcriptional activation by E47 and its derivative.

Diagrams of E47 and E-T/2 proteins are shown on the top. The E47 sequence is designated as hatched boxes and the Tal1 sequence are represented as dotted boxes. T47D and MDA-MB-231 cells were transfected with 5 μg E-box luciferase reporter with 4 μg of each indicated expression plasmid. The control sample contains equal amount of the vector DNA (pcDNA3). Two μg of a pCMV-lacZ plamsid was also co-transfected in each sample. Thirty-six hours post transfection, luciferase and β-galactorsidase acitivities in each transfected cells were measured. Luciferase activites were first normalized against β-galactosidase activities. The corrected activities were then compared with the control sample to obtained the relative activity as shown in the Y axis. Log scale is used for the Y axis.

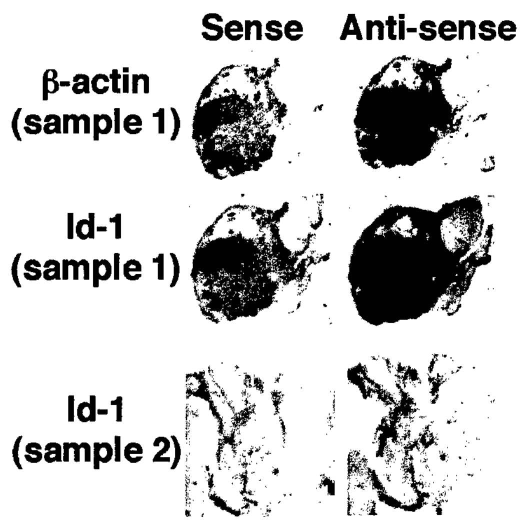


Figure 5. In situ RNA hybridization.
Invasive adenocarcinoma specimens were from NYU breast cancer archive. Five micrometer sections were used to hybridize with ^{35}S-labeled sense or antisense RNA probes for β-actin or Id-1. After hybridization and washing, the signals were detected by using phosphoImager. Two samples with different levels of Id-1 expression are shown.

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Curriculum Vitae

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EDUCATION:

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1987 - 1988	Whitehead Institute for Biomedical Research, Mentor: Dr. David Baltimore, Field: protein synthesis inhibition by poliovirus and anti-HIV strategies.
1989 - 1991	Whitehead Institute for Biomedical Research, Mentor: Dr. David Baltimore, Field: structure and function of basic helix-loop-helix transcription factors.

ACADEMIC APPOINTMENTS:

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1991 - 1998	Reviewer for "Journal of Virology", "Molecular and Cellular Biology", "Nucleic Acids Research", "Journal of Cellular Physiology", Journal of Neuroscience" and 'Journal of Immunological Methods".
1993	Reviewer for grants received by the United States Army Medical Research and Development Command for research on breast cancer.
1994 - 1998	Seminar speaker at NIA, Thomas Jefferson Cancer Center, Harvard

	Medical School, Yale Medical School, NCI, UCLA and UMDNJ.
1995	Invited speaker at the Sixth International Symposium of the Society of Chinese Bioscientists in America, Vancouver, Canada.
1995 -1997	Reviewer and site-visitor for two NIH (NIDDK) program project grants.
1996	Speaker at the Joint Symposium of American Association of Immunologists, American Association of Biochemists and American Associate of Investigative Pathologists, New Orleans, LA.
1997	Invited speaker at the Seventh International Symposium of the Society of Chinese Bioscientists in America, Toronto, Canada
1998	Invited speaker at Gene Therapy/Molecular Biology International Conference, Heraklion island of Crete, Greece.
1998	Invited speaker at New England Immunology Conference, Woods Hole, MA.
1999	Invited speaker at Gene Therapy/Molecular Biology International Conference, Redwood City, CA.

AWARDS:

1981	CUSBEA (China-United States Biochemistry Examination and Application) exchange student.
1989 - 1991	Cancer Research Institute Postdoctoral Fellowship.
1991 - 1996	Markey Scholar (Supported by a grant from The Lucille P. Markey Charitable Trust Foundation to NYUSM).
1992 - 1993	Institutional Whitehead Presidential Fellowship.
1992 - 1996	Cancer Research Institute Investigator Award.
1994 - 1998	Irma T. Hirschl Trust Career Scientist Award.

PROFESSIONAL SOCIETIES:

1991	Member of the American Association for the Advancement of Science.
1992	Member of the American Society for Microbiology.
1994	Member of the Society of Chinese Bioscientists in America. Served in the Council (1996) and the Membership Committee (1994-1996) of Society of Chinese Bioscientists in America. President of the Tri-State Chapter (1997-1998).
1998	Member of Ray Wu Society for Life Sciences. Board of the Directors (1998-2000).

PAST MAJOR GRANTS:

1991 - 1997	The Lucille P. Markey Charitable Trust Foundation. "Molecular mechanisms of Hematopoiesis"

	Total direct cost $511,875.
1992 - 1994	American Cancer Society Research Grant. "Regulation of the Id genes in B cell development". Total direct cost $146,380.
1992 - 1997	Cancer Research Institute Investigator Award. "Role of the HLH Id proteins in B lymphoid differentiation and neoplasia". Total direct cost $200,000.
1994 - 1998	Irma T. Hirschl Trust Career Scientist Award. "Molecular mechanisms of B cell differentiation". Total direct cost $100,000.

ACTIVE GRANTS

1994 - 1999	NIH R01 Grant from NIAID. "Regulation of the Id genes in B cell development". Total direct cost $397,046. (Re-newal pending)
1996 - 1999	Life and Health Insurance Medical Research Fund Grant "Regulation of B cell differentiation by the E2A transcription factor". Total direct cost $75,000.
1998 - 2001	US Army "The role of Id proteins in breast cancer." Total direct cost $210,000.
1998 - 2001	American Cancer Society The molecular mechanism of T cell leukemogenesis induced by TAL. Total direct cost $300,000 (relinquished)
1998 - 2003	NIH R01 Grant from NCI. The molecular mechanism of T cell leukemogenesis induced by TAL. Total direct cost $915,144.

TEACHING EXPERIENCE:

Graduate and Medical School Courses:

1992	Nucleic Acids Core Course for graduate students.
1992	Molecular and Cellular Biology
1994 - 1998	Cell Biology of tissues and organs (Histology).

Graduate School Training:

1991 - 1998	New York University School of Medicine.

Thesis advisor: Four graduate students and one MD-PhD student. (three students received Ph.D.).
Thesis committee member: Five graduate students and one MD-PhD students.

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2. Tso, J. Y., **Sun, X.-H.** and Wu, R. (1985) Structure of two unlinked *Drosophila melanogaster* glyceraldehyde 3-phosphate dehydrogenase genes. *J. Biol. Chem.* 260: 8220-8228.

3. **Sun, X.-H.,** Lis, J. and Wu, R. (1988) The positive and negative transcriptional regulation of the *Drosophila Gapdh*-2 gene. *Genes Dev.* 2: 743-753.

4. **Sun, X.-H.**, Tso, J. Y., Lis, J. and Wu, R. (1988) Differential regulation of the two GAPDH genes during *Drosophila* development. *Mol. Cell. Biol.* 8: 5200-5205.

5. **Sun, X.-H.**, and Baltimore, D. (1989) Human immunodeficiency virus tat-activated expression of poliovirus protein 2A inhibits mRNA translation. *Proc. Nalt. Acad. Sci. USA* 86: 2143-2146.

6. Kamps, M. P., Murre, C., **Sun, X.-H.**, and Baltimore D. (1990) A new homeobox gene contributes the DNA-binding domain of the t(1:19) translocation protein in pre-B ALL. *Cell* 60: 547-555.

7. **Sun, X.-H**. and Baltimore, D. (1991) An inhibitory domain of E12 prevents DNA binding in E12 homodimers but not in E12 heterodimers. *Cell* 64: 459-470.

8. **Sun, X.-H.**, Copeland, G., Jenkins, N. and Baltimore, D. (1991) The Id proteins, Id1 and Id2, selectively inhibit DNA binding by one class of bHLH transcription factors. *Mol. Cell. Biol.* 11: 5603-5611.

9. **Sun, X.-H.** (1994) Constitutive expression of the Id1 gene impairs mouse B cell development. *Cell* 79: 893-900.

10. Saisanit, S. and **Sun, X.-H.** (1995) A novel enhancer, PBE, regulates Id1 gene expression in progenitor B-cells. *Mol. Cell. Biol.* 15: 1513-1521.

11. Laure, T. M., Starovasnik, M. A., Weintraub, H., **Sun, X.-H.** and Klevit, R. E. (1995) MyoD forms micelles which can dissociate to form heterodimers with E47. Implications of micellization on function. *Natl. Proc. Acad. Sci. USA.* 92: 11824-11828.

12. Mahajan, M. A., Park, S. T., and **Sun, X.-H.** (1996) Association of a novel GTP-binding protein, DRG, with TAL oncogenic proteins. *Oncogene* 12: 2343-2350.

13. Vitola, S. J., Wang, A. and **Sun, X.-H.** (1996) Substitution of basic amino acids in the basic region stabilizes DNA binding by E12 homodimers. *Nucl. Acid. Res.* 24:1921-1927.

14. Saisanit, S. and **Sun, X.-H.** (1997). Regulation of the pro-B cell specific enhancer of the Id1 gene involves the C/EBP family of proteins. *Mol. Cell Biol.* 17: 844-850.

15. Chen, B., Han, B. H., **Sun, X.-H.** and Lim, R. W. (1997). Inhibition of muscle-specific gene expression by HLH462/Id3: requirement of the C-terminal region of the protein for stable expression and function. *Nucl. Acid. Res.* 25: 423-430.

16. Prabhu, S., Ignatova, A., Park, S. T. and **Sun, X.-H.** (1997). Regulation of the expression of cyclin-dependent kinase inhibitor, p21, by E2A and Id proteins. *Mol. Cell. Biol.* 17: 5888-5896.

17. Dang, W., **Sun, X.-H.** and Sen, R. (1998). ETS mediated cooperation between bHLH motifs of the immunoglobulin μ heavy chain gene enhancer. *Mol. Cell. Biol.* 18: 1477-1488.

18. Park, S. T. and **Sun, X.-H.** (1998). The Tal1 Oncoprotein inhibits E47-mediated transcription: Mechanism of Inhibition. *J. Biol. Chem.* 273: 7030-7037.

19. Park, S. T., Nolan, G. P. and **Sun, X.-H.** (1999). Growth inhibition and apoptosis due to restoration of E2A activity in T-ALL cells. *J. Exp. Med.*,189: 501-508.

20. Pan, L., Sato, S., **Sun, X.-H.** and Zhuang, Y. Impaired immune responses and B cell proliferation in mice lacking the Id3 gene. (submitted to Mol. Cell. Biol.).

21. Yao, Y., Doki, Y., Jiang, W., Venkatraj, V. S., Warburton, D., Santella, R. M., Lu, B., Yan, L., **Sun, X.-H.**, and Weinstein, I. B. Interactive cloning and characterization of *dip1*, a novel cDNA encoding a cyclin-D1-binding protein that is related to the Id family of proteins. (submitted to Mol. Cell. Biol.).

22. Kim, D., Peng, X., and **Sun, X.-H.** 1999. Id1 overexpression in transgenic mice causes T cell developmental block, apoptosis and lymphoma (Mol. Cell. Biol., in revision).